[Plant Signaling & Behavior 2:4, 293-295; July/August 2007]; ©2007 Landes Bioscience

Article Addendum

Phyllotaxy

Beyond the Meristem and Auxin Comes the miRNA

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Original manuscript submitted: 02/20/07
Manuscript accepted: 02/20/07

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/4040

KEY WORDS

phyllotaxy, CUP-SHAPED COTYLEDON, boundary domain, internode, miRNA

ACKNOWLEDGEMENTS

This work was partially supported by an ACI Jeunes Chercheurs award to P.L. and the trilateral Génoplante GENOSOME program TRIL-046.

Addendum to:

Plants Expressing a miR164-Resistant CUC2 *Gene Reveal the Importance of Post-Meristematic Maintenance of Phyllotaxy in Arabidopsis*

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Development 2007; 134:1045–50
PMID: 17251269
DOI: 10.1242/dev.02774

ABSTRACT

Phyllotaxy, the arrangement of organs along the stem, has puzzled scientists for centuries. The shoot apical meristem plays a crucial role in the formation of this pattern, by initiating organ primordia on its flanks in a temporally and spatially controlled manner. Recent studies have shown that primordium position at the meristem is governed by local auxin gradients, but little is known about the subsequent events leading to the phyllotaxy along the mature stem.

In a recent report we showed that deviation from the initial phyllotaxy set-up in the meristem is generated during stem growth of transgenic lines affected in *miR164*-mediated regulation of *CUC2* and, to a smaller extent, of wild-type Arabidopsis. This underlines the requirement of maintaining the pattern initiated at the meristem during stem development. In this addendum, we discuss the importance of this mechanism in different mutants and at different stages of Arabidopsis development.

Lateral organs such as leaves are arranged along the stem following a regular pattern called phyllotaxy.[1] The spiral phyllotaxy where the organs follow a regular generative spiral running along the stem is the most common in plants, but other patterns such as whorled or opposite phyllotaxy are observed too. Lateral organs are initiated at the tip of the stem by a group of undifferentiated, proliferating cells called the shoot apical meristem (SAM), in a regular pattern leading to the setting up of the phyllotaxy.[2] Recent evidences show that local distribution of auxin controls the site of primordium initiation and its outgrowth.[1]

Proper initiation of the organ primordia requires the formation of boundary domains that separate these structures from either other or from the meristem. In Arabidopsis, the *CUC1*, *2* and *3* (*CUP-SHAPED COTYLEDON1, 2* and *3*) genes are expressed in- and define the identity of the boundary domain.[3-5] Inactivation of one or several of these members of the NAC family of transcription factors leads to fusion between neighbouring organs, suggesting that these genes locally repress proliferation. Supporting this, *CUC* genes show complementary expression with cell proliferation markers during Arabidopsis flower development.[6] More recently, *CUC2* was shown to have a similar role in growth repression during the formation of leaf serration.[7] *CUC1* and *CUC2* transcripts are targeted for endonucleolytic cleavage by the microRNA *miR164*.[8,9] This post-transcriptional regulation has been shown to be important during the development of the leaf and the flower.[7,10-12] We recently observed that plants expressing *CUC2g-m4*, a *CUC2* gene engineered to make it resistant to *miR164* show a dramatic perturbation of the phyllotaxy.[13] Wild-type Arabidopsis show a typical spiral phyllotaxy. In contrast, *CUC2g-m4* plants show a more variable phyllotaxy with formation of clusters of flowers, with many short internodes and occasional long internodes. In contrast to wild type, the divergence angle between successive organs appears to be random.

We investigated the causes of these changes of phyllotaxy. As the phyllotaxy is set up in the meristem, we investigated this structure in *CUC2g-m4* plants. In contrast to several mutants in which an abnormal phyllotaxy is associated with a change in meristem size and/or organisation,[14-17] we found no evidence for this in *CUC2g-m4* apices. That meristem function *per se* was essentially unperturbed in these transgenic lines was further confirmed by the observation of normal picks of auxin response associated with primordium formation as judged from the auxin response reporter *DR5:GFP*. Finally, we measured the divergence angles between successive primordia in the meristem of *CUC2g-m4* plants and found them to be identical to those observed in the wild type. All together, this shows that primordia formation occurs normaly in *CUC2g-m4* plants and that the phyllotaxy defects observed in mature stem occurs below the meristem while the stem is growing.

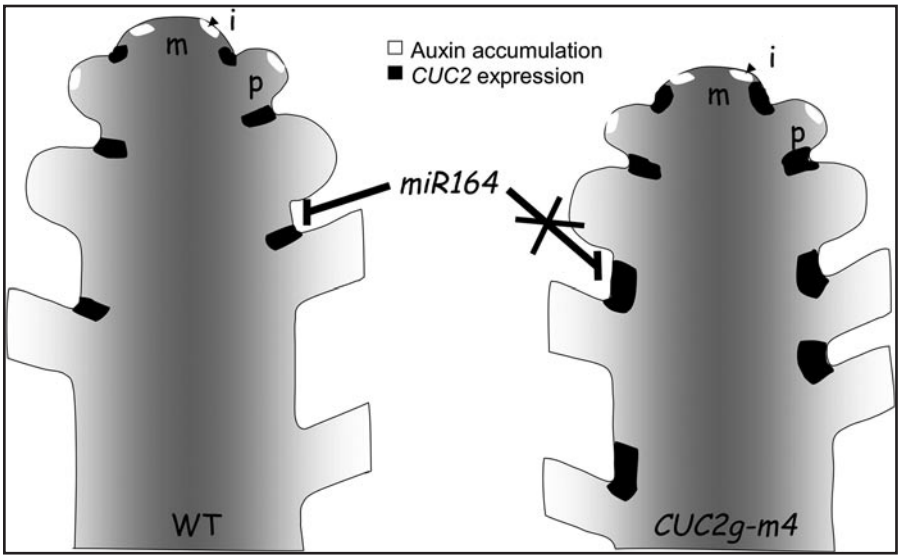

Figure 1. *miR164* is required for the maintenance of the phyllotaxy via the regulation of *CUC2* expression in the developing stem. In wild type plants (WT, left panel), phyllotaxy is generated in the meristem (m). The initia (i) are positioned by local auxin accumulation. This pattern is conserved during primordia outgrowth (p) and organ development. During stem growth, *CUC2* expression is restricted to the axillary domain. Interfering with *miR164* regulation of *CUC2* leads to dramatic changes of the phyllotaxy (*CUC2g-m4*, right panel). Auxin accumulation and primordia positions in the meristem are not affected whereas internode growth is modified. This modification is correlated with maintenance of *CUC2* expression in the internode.

In order to further characterise the defects leading to the abnormal phyllotaxy defects, we analysed the cell numbers and sizes along the internodes. Changes in internode lengths are due to a combination of abnormal cell sizes and numbers. To relate these modifications to the primary effect triggered by the *CUC2g-m4* construct, we investigated by in situ hybridisation the expression of *CUC2* in longitudinal section of wild type and transgenic plants. In wild type apices, *CUC2* is expressed in a narrow domain corresponding to the boundary domain. In the developing stem, *CUC2* mRNA is rapidly cleared from the elongating internode and becomes restricted to the axils of the floral pedicels. *CUC2g-m4* plants showed stronger and enlarged expression of *CUC2*. In particular, *CUC2* expression was detected in the internode region. Given the proposed role of the CUC2 protein in the regulation of growth, we suggest that ectopic expression of CUC2 during internode development interferes with normal growth, leading to the alteration of the phyllotaxy set up in the meristem (Fig. 1).

This work raised the question whether such a mechanism at the basis of the variation of the phyllotaxy is limited to the *CUC2g-m4* transgenics or whether it could occur in different lines and what could it tell us about development in wild type.

Phyllotaxy defects have been reported for mutants affected in the miRNA pathway. Mutation of the *HEN1* gene, known also as *CRM2* that is required for miRNA methylation leads to defects in internode elongation.[18-20] Phyllotaxy defects are also observed when the *SE* gene that is required for miRNA precursor maturation is inactivated.[21-23] In addition, we observed phyllotaxy defects in the *hyl1-1* and *dcl1-9/caf* mutants that affect miRNA precursor maturation.[24-26] Although these mutants are highly pleiotropic as many if not all of their miRNA are affected, one could speculate that their phyllotaxy defects are at least partially due to a similar mechanism as the one revealed by the *CUC2g-m4* line. Consistent with this hypothesis, some of these mutants accumulate *CUC2* transcripts at a higher level and show enlarged boundary domains in the flowers.[9,11,24]

Our model also predicts that reduced *miR164* accumulation should lead to phyllotaxy defects. *miR164* is encoded by three genes in Arabidopsis *MIR164A*, *MIR164B* and *MIR164C*. Mutation in any of these genes does not lead to an abnormal phyllotaxy.[7,10,12] Therefore, they may redundantly contribute to the regulation of *CUC2* expression, an hypothesis recently confirmed.[27]

Finally, we precisely analysed phyllotaxy in the wild type. The distribution of the divergence angles between successive primordia was much sharper in the meristem then along the mature stem (Fig. 2). This shows that variability of the phyllotaxy initiated at the meristem is generated during wild type stem development, although we have so far no indication that *CUC2* is involved in that. Interestingly, the distribution of the divergence angles is much sharper during the vegetative phase where no internode elongation occurs then during reproductive development (Fig. 2), further linking the variability of the phyllotaxy with stem growth. All together our work shows that the phyllotaxy does not only rely on meristem function but also on later steps of stem growth and differentiation.

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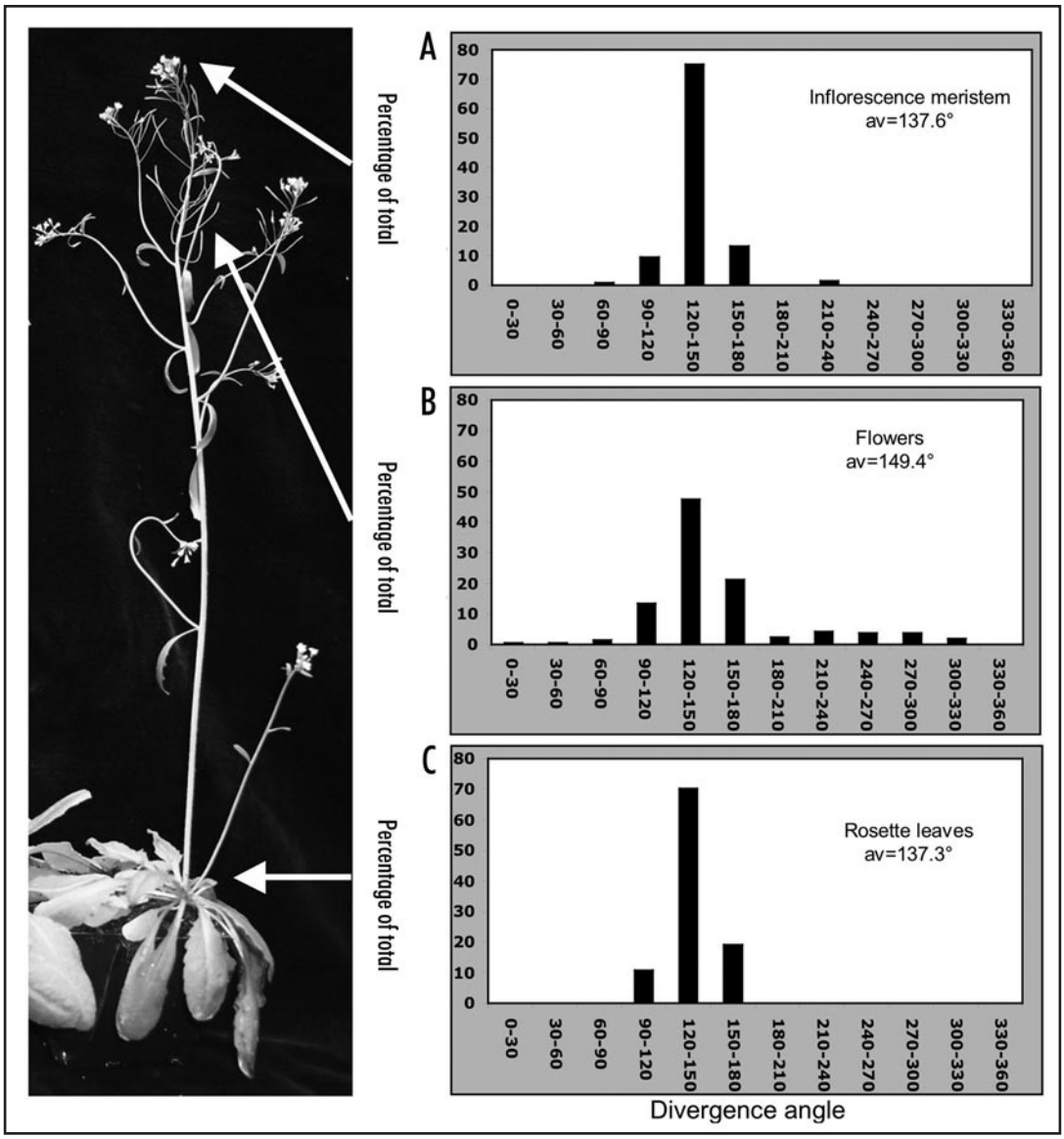

Figure 2. Variability of the phyllotaxy during the different growth stages of wild-type Arabidopsis. (A) In the inflorescence meristem, the phyllotaxy is only slightly variable as represented by the distribution of the divergence angles between two successive organs. (B) In contrast, the phyllotaxy is more variable when measured for the mature organs along the fully-grown stem. Therefore, the variability of the phyllotaxy is increased during stem development. (C). The phyllotaxy is less variable during the vegetative than during the reproductive phase, as shown by the distribution of the divergence angles between successive rosette leaves. The average value (av) of the divergence angles is indicated.

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